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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/211,691	12/14/1998	MICHEL GILBERT	14137-129-10	9572

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EXAMINER	
RAO, MANJUNATH N	

ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 05/06/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/211,691	GILBERT ET AL.	
	Examiner	Art Unit	
	Manjunath N. Rao, Ph.D.	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *03 February 2005*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *37-48* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *37-48* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *2-3-05*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

CONTINUED EXAMINATION UNDER 37 CFR 1.114 AFTER APPEAL BUT BEFORE A BOARD DECISION

A request for continued examination under 37 CFR 1.114 was filed in this application after appeal to the Board of Patent Appeals and Interferences, but prior to a decision on the appeal. Since this application is eligible for continued examination under 37 CFR 1.114 and the fee set forth in 37 CFR 1.17(e) has been timely paid, the appeal has been withdrawn pursuant to 37 CFR 1.114 and prosecution in this application has been reopened pursuant to 37 CFR 1.114. Applicant's submission filed on 2-3-05 has been entered.

Claims 37-48 are currently pending and are present for examination.

Applicants' amendments, arguments and declaration under 37 C.F.R. 1.132 filed on 2-3-05, have been fully considered and are deemed to be persuasive to overcome the rejections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 37-48 rejected under 35 U.S.C. 103(a) as being unpatentable over Bulow et al. (TIBtech, 1991, Vol. 9:226-231), Defrees et al. (WO 96/32491), Gilbert(a) et al. (Eur. J. Biochem., 1997, Vol. 187:187-194) and Gilbert(b) et al. (Biotech. Lett., 1997, Vol. 19(5):417-

420) and the common knowledge in the art of molecular biology provided by Sambrook et al. (Molecular Cloning, A Laboratory Manual, 2nd Ed, ColdSpring Harbor Laboratory Press, 1989, pages 7.37-7.52), . Claims 37-48 are drawn to an polynucleotide encoding a fusion polypeptide comprising a bacterial, i.e., a Neisserial, α 2,3-sialyltransferase and CMP-Neu5Ac synthetase, comprising a signal sequence and a molecular tag wherein the two polypeptides are linked through a peptide linker, an expression vector comprising said polynucleotide, a host cell expressing said vector, a method of making said polypeptide by growing the host cells and followed by purification of the fusion polypeptide and permeabilizing the host cell.

Bulow et al. teach the value of artificial bi-functional enzyme as well as multienzyme systems obtained by gene fusion. The reference teaches that preparation of bi and multi-functional enzymes by gene fusion has a great potential in enzyme technology as they facilitate easy purification and exhibit favorable enzyme kinetics. The reference also teaches that selective enzymes can be made as fusion enzymes and used in biochemical analysis, enzyme process technology and metabolic engineering. However the reference does not teach specifically the making of a fusion polynucleotide encoding a fusion protein comprising a sialyltransferase and sialic acid synthetase.

Defrees et al. teach the enzymatic synthesis of glycosidic compounds such as sialic acid compounds using individual enzymes such as a glycosyltransferase, a sialyltransferase and a CMP-NeuAc synthetase for generation of sialic acid and CTP (see entire document and specifically claims 1-10). The reference teaches in detail the other requirements for the enzymatic synthesis of carbohydrate compounds involving multiple enzymes. However, while the reference teaches the use of each individual enzymes isolated by recombinant or natural

sources the reference does not teach the use of gene fusion either for making these multiple enzymes.

Sambrook et al. provide an exhaustive volume of methods that can be used for various gene manipulations including making fusion polynucleotides, introduction of linker sequences and use of tag sequences in recombinant proteins for easy purification. The reference also teaches purification of recombinant proteins using the molecular tags associated with such proteins. Techniques such as permeabilizing cells for easy access of the encoded enzymes to the substrates are also well k known in the art.

Gilbert(a) et al. teach the characterization of a recombinant *Neisseria* α 2,3-sialyltransferase which plays an important role in the transfer of sialic acid from CMP-NANA to acceptor oligosaccharides which in turn plays a role in cell-cell recognition. The reference also teaches that investigation of the enzymology of glycosyltransferase involved in LOS biosynthesis is limited due to the lack of bacterial glycosyltransferase. The reference provides the amino acid sequence of the enzyme from which a cDNA clone can be developed.

Gilbert(b) et al. teach the purification and characterization of the recombinant CMP-sialic acid synthetase (CSA) from *Neisseria* and teach its use coupled with α 2,3-sialyltransferase(ST) to synthesize CMP-sialic acid which is further attached to various biopolymers. The reference teaches that the major application of the CSA is in "coupled reactions" with sialyltransferases to sialylate oligosaccharides using CTP and NANA as substrates instead of CMP-Neu5Ac which is relatively unstable and expensive. The reference teaches the use of CSA and ST in a coupled reaction to sialylate FCHASE-lactose. The reference concludes that CSA enzyme works effectively in a coupled reaction with a ST. The reference also lists several advantages of CSA.

However, the reference does not teach the use of fusion polynucleotide comprising encoding sequences of both the above enzymes.

Combining the teachings of the above references it would have been obvious to one of ordinary skill in the art to make a single bi-functional fusion enzyme as taught by Bulow et al. using the CSA and SA taught by Gilbert et al. references. One of ordinary skill in the art would have been motivated to do so in order to develop a one-pot synthesis of FCHASE-lactose. One of ordinary skill in the art would be motivated to make a fusion protein or a host cell comprising a polynucleotide expressing said fusion protein (comprising a Neisseria CSA and ST as taught by Gilbert et al.) because the reference teaches a method involving the use of the two enzymes in the same vessel and that the synthetase works effectively in a coupled reaction with ST. Those skilled in the art would be motivated to permeabilize such host cells using well known methods in the art, so that the enzymes become easily accessible for the acceptor and donor substrates, for direct use of such host cells in sialylating reactions. Furthermore, such a method would obviate the use of expensive and unstable CMP-Neu5Ac from an external source as it is generated *in situ* and used immediately in the sialylation step. One of ordinary skill in the art would have a reasonable expectation of success since the Gilbert references teach both the enzymes and their compatibility in synthesizing FCHASE-lactose, Bulow et al. teach the increasing use of bi-functional enzymes and Sambrook et al. teach methods for developing fusion protein encoding polynucleotide and methods of making recombinant protein.

Therefore the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art.

In response to the previous Office action, applicants have traversed the above rejection continuing their previous argument that none of the cited references disclose all the elements of the claimed invention and also fail to provide motivation for combination of the references to arrive at the claimed invention. Examiner respectfully disagrees with such an argument for the very same reasons that he set aside in the previous Office action.

Applicants maintain the "unexpected results" argument. In support of such an argument applicants have filed a declaration under 35 U.S.C. 1.132. Applicants argue that, with regard to the improvement in individual enzymatic activity, Bulow et al. teach only improvements in a coupled reaction activities, relying on for example proximity effects for the difference between unfused enzymes and fused enzymes. Applicants argue Bulow et al. does not teach improvement in activity of individual proteins after fusion and quote from Bulow et al. "when corrected for the increase in molecular weight caused by the fusion, the specific activities correspond to 50-100% of those of native enzymes" and in contrast to that the instant specification demonstrates that the turnover rate of the unfused α 2,3-sialylîansferase is 1.4 s^{-1} while the turnover rate of the fused α 2,3-sialyltransferase is 3.2 s^{-1}. Applicants continue such argument arguing that because of this difference the activity of the fused α 2,3-sialyltransferase corresponds to 229% of the activity of the unfused enzyme. Applicants provide similar numbers for the synthetase enzyme and argue that since Bulow et al. did not teach any improvement in specific activity of an individual protein on fusion, Dr. Gilbert asserts that the observed increases of 129% of α 2,3-sialyltransferase activity and 26% of CMP-NeUAC synthetase activity are unexpected. Examiner respectfully disagrees with such an argument. This is because, applicants' argument that Bulow et al. teach only improvements in a coupled

reaction activities, relying on for example proximity effects for the difference between unfused enzymes and fused enzymes and as if their invention does not depend on the above factors taught by Bulow et al. (i.e., proximity effects) is highly misplaced. The reference of T.G. Warner (Nature Biotechnol. 16:720-721, 1998, which reviews applicants' invention) provided by the applicants teaches that "the proximity of the two microbial enzymes fortuitously results in improved physical and chemical properties" (page 721, col.1) which is the same as that taught by Bulow et al. This clearly shows that applicants' invention also depends on the proximity of the two enzymes. Next, Examiner takes the position that applicants are comparing two different properties of the fused proteins when comparing their invention with that of the teaching of Bulow et al. Applicants compare the *turnover number* of their invention with the teaching of the *specific activity* of Bulow et al. These are two different characteristics of a protein or a fused protein. *Specific activity* is defined as a term useful for describing the purity and activity of an enzyme and expressed as *enzyme units per milligram of total protein*, while the *turnover number* , a term that is occasionally used in modern biochemistry is equivalent to "*molecular activity*" defined as *enzyme units per micromole of the enzyme*. Therefore these are two distinct ways of describing a protein and are not the same characteristics. The *specific activity* explained by Bulow et al. takes into consideration the total protein while the *turnover number* does not. Therefore, contrary to applicant's argument it cannot be concluded that the instant *turnover numbers* are unexpected because, this is an inherent characteristic of the fusion protein not addressed by Bulow et al. in their teachings.

The reference of T.G Warner (Nature Biotechnol. 16:720-721, 1998) also refers to the gain in *turnover number* as a "modest improvement" (column 1, page 721) and that "the fusion

protein had kinetic properties that were similar, if not slightly improved over the two separate enzymes" (column 2, page 721). In light of such evaluations by those skilled in the art, Examiner takes the position that the results are not unexpected than that already taught by Bulow et al.

Furthermore, Examiner also notes that the *turnover numbers* provided by the applicants apply to a single example of a fusion protein comprising a specific CMP-sialic acid synthetase isolated from *Neisseria* and a α 2,3-sialyltransferase isolated from *E.coli*. Applicants have not provided any evidence to suggest that such would be the case with a fusion protein comprising enzymes with identical activities from any or all sources. In view of the absence of such evidence, it cannot be agreed that the results are unexpected in all cases.

While Examiner agrees that applicants have indeed shown that the solubility of one of the enzymes increased to five folds compared to the unfused enzyme, he respectfully disagrees that applicants have submitted evidence of substantially improved results that were unexpected. For all the above reasons Examiner continues to maintain the above rejection.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103(a).

Conclusion

None of the claims are allowable.

This is a continuation of applicant's earlier Application No. 09/211691. All claims are drawn to the same invention claimed in the earlier application and could have been finally rejected on the grounds and art of record in the next Office action if they had been entered in the earlier application. Accordingly, **THIS ACTION IS MADE FINAL** even though it is a first action in this case. See MPEP § 706.07(b). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no, however, event will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Manjunath N. Rao, Ph.D. whose telephone number is 571-272-0939. The Examiner can normally be reached on 7.00 a.m. to 3.30 p.m. If attempts to reach the examiner by telephone are unsuccessful, the Examiner's supervisor, Ponnathapura Achutamurthy can be reached on 571-272-0928. The fax phone numbers for the organization

where this application or proceeding is assigned is 703-872-9306 for regular communications and for After Final communications. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 571-272-1600.

Manjunath N. Rao Ph.D.
Patent Examiner, A.U. 1652
4/27/05